lipid N-[1-(2,3-dioleyloxy)propyl]-N,N,N-trimethylammonium chloride and dioleyl phosphatidylethanolamine (adding up to 1 mg/ml) in membrane filtered water; GIBCO BRL Life Technologies Inc., Gaithersburg, USA); °Lipofectamine (a 3:1 (w/w) liposome formulation of the polycationic lipid 2,3-dioleyloxy-N-[2-(sperminecarboxyamido)ethyl]-N,N-dimethyl-1-propanaminium trifluoroacetate (DOSPA) and the neutral lipid dioleoyl phosphatidylethanolamine (DOPE) in membrane filtered water (GIBCO BRL, USA); N-[1-(2,3-Dioleoyloxy)-propyl]-N,N,N-trimethylammoniumsulfate (Boehringer Mannheim GmbH, FRG), which are present preferably in concentrations ranging from about 0.2 to about 20 µg/ml, for example about 5 µg/ml, in the respective in vitro experiments.

The present invention relates also to pharmaceutical compositions comprising an oligonucleotide or an oligonucleotide derivative with the properties according to the invention as active ingredient. Especially preferred are compositions for enteral, especially oral, or parenteral administration. The compositions comprise the active ingredient on its own or, preferably, together with a pharmaceutically acceptable carrier. The dose of the active ingredient depends on the disease to be treated, and on the species, age, weight and individual condition, as well as the method of administration.

Preferred is a pharmaceutical composition that is suitable for administration to a warm-blooded animal, especially man, suffering from a disease that responds to the modulation of PSA synthesis; for example a proliferative and especially hyperproliferative disease, preferably a tumor disease, especially a leukemia; a tumor of the prostate, such as prostatic carcinoma; a tumor of the colon; a brain tumor; a hyperproliferative skin or epithelial disease, for example psoriasis; a tumor of the epidermis, such as melanoma; (preferably) a lung cancer, such as lung small-cell carcinoma; and/or (most preferably) a tumor of the urinary tract, especially bladder carcinoma; and any metastases derived therefrom; comprising an amount of the active ingredient, or of a salt thereof if salt-forming groups are present, that is effective in the modulation of the synthesis of PSA, preferably in the treatment or prophylaxis of the mentioned diseases, together with at least one pharmaceutically acceptable carrier.

The pharmaceutical compositions comprise from approximately 0.0001 % to approximately 95% active ingredient, dosage forms that are in single dose form preferably comprising from

approximately 0.001 % to approximately 20 % active ingredient, and dosage forms that are not in single dose form preferably comprising from approximately 0.001 % to approximately 10 % active ingredient. Unit dose forms, such as dragées, tablets, ampoules or capsules, comprise from approximately 0.0005mg to approximately 0.5 g of the active ingredient, preferably from 0.005 mg to approximately 20 mg.

The pharmaceutical compositions of the present invention are prepared in a manner known *per se*, for example by means of conventional mixing, granulating, confectioning, dissolving or lyophilising processes. For example pharmaceutical compositions for oral administration can be obtained by combining the active ingredient with one or more solid carriers, where necessary granulating a resulting mixture and processing the mixture or the granules, if desired or appropriate with the addition of further excipients, to form tablets or dragée cores.

Suitable carriers are especially fillers, such as sugars, e.g. lactose, saccharose, mannitol or sorbitol, cellulose preparations and/or calcium phosphates, e.g. tricalcium phosphate or calcium hydrogen phosphate, and binders, such as starches, e.g. corn, wheat, rice or potato starch, methylcellulose, hydroxypropylmethylcellulose, sodium carboxymethyl-cellulose and/or polyvinylpyrrolidone, and/or, if desired, disintegrators, such as the above-mentioned starches, and also carboxymethyl starch, crosslinked polyvinylpyrrolidone or alginic acid or a salt thereof, such as sodium alginate. Additional excipients are especially flow conditioners and lubricants, e.g. silicic acid, talc, stearic acid or salts thereof, such as magnesium or calcium stearate, and/or polyethylene glycol, or derivatives thereof.

Dragée cores may be provided with suitable, optionally enteric, coatings, there being used, *inter alia*, concentrated sugar solutions which may comprise gum arabic, talc, polyvinyl-pyrrolidone, polyethylene glycol and/or titanium dioxide, or coating solutions in suitable organic solvents or solvent mixtures, or, for the preparation of enteric coatings, solutions of suitable cellulose preparations, such as acetylcellulose phthalate or hydroxypropylmethyl-cellulose phthalate. Dyes or pigments may be added to the tablets or dragée coatings, e.g. for identification purposes or to indicate different doses of active ingredient.

Orally administrable pharmaceutical compositions are also dry-filled capsules consisting of gelatin, and also soft sealed capsules consisting of gelatin and a plasticiser, such as

20. A hybrid vector comprising an isolated nucleic acid according to claim 11 which nucleic acid is operably linked to suitable control sequences.

21. A host cell transfected with a hybrid vector according to claim 20.

22. A host cell capable of producing a protein according to claim 1 and containing a heterologous nucleic acid encoding said protein.

23. A method for identifying a compound capable of binding to PSA, said method comprising employing a protein according to claim 1 or 3 in a binding assay.

24. A method for identifying a compound or agent which modulates the biological activity of PSA, said method comprising contacting a protein according to any of claims 1 or 3 with at least one compound or agent, whose ability to modulate the activity of PSA is sought to be investigated, and determining the change of PSA activity of said protein caused by the component or agent.

25. A method according to claim 24 comprising contacting cells producing functionally active PSA and containing heterologous DNA encoding PSA with at least one compound to be tested for its ability to modulate the activity of PSA, and monitoring said cells for a resulting change in PSA activity.

26. A method of inducing apoptosis in a cell comprising introducing into said cell a compound or signal directly or indirectly interfering with PSA activity.

27. A method of reducing the viability of a proliferating mammalian cell or cell population exhibiting PSA activity comprising down regulating the expression level or substantially inhibiting the activity of PSA in the proliferating cell or cell population.

28. A compound inducing apoptosis in a cell which compound specifically modulates the activity of PSA in said cell and which is identified by the method of claim 25.

29. A compound reducing the viability of a proliferating mammalian cell or cell population exhibiting PSA activity which compound specifically modulates the activity of PSA in said cell and which is identified by the method of claim 25.

30. A method of modulating the expression of PSA comprising contacting tissues or cells containing the PSA gene with an oligonucleotide or oligonucleotide derivative comprising from 5 to 50 nucleotide units specifically hybridizable with selected DNA or RNA deriving from the PSA gene.

31. A method of diagnosing conditions associated with PSA expression comprising contacting cells or tissues or body fluids from an animal suspected of having a condition associated with PSA expression with an oligonucleotide or an oligonucleotide derivative, or a salt thereof where salt-forming groups are present, specifically hybridizable with selected DNA or RNA deriving from the PSA gene, and determining whether hybridization occurs.

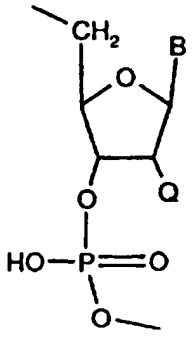
32. An oligonucleotide or an oligonucleotide derivative, or a salt thereof where salt-forming groups are present, which is specifically hybridizable with RNA deriving from the gene that encodes functional PSA, particularly PSA with the amino acid sequence set forth in SEQ ID NO:6, and preferably PSA with the amino acid sequence set forth in SEQ ID NO:2.

33. An oligonucleotide or an oligonucleotide derivative, or a salt thereof where salt-forming groups are present, according to claim 32 that is specifically hybridizable with RNA deriving from the gene that encodes PSA, comprising analogues of nucleotide units sufficient in number and identity to allow such hybridization, or a salt of said oligonucleotide derivative where salt-forming groups are present.

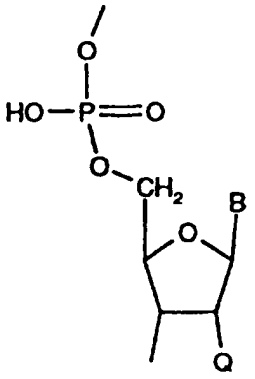
34. An oligonucleotide or oligonucleotide derivative, or a salt thereof where salt-forming groups are present, according to claim 32, which is specifically hybridizable with a particular target sequence identified in Example 3 with respect to the nucleic acid sequence set forth in SEQ ID NO:1.

35. An oligonucleotide or oligonucleotide derivative according to claim 32 comprising 5 to 50 nucleotide units.

36. An oligonucleotide or oligonucleotide derivative, or a salt thereof where salt-forming groups are present, according to claim 32 with any of the sequences given in SEQ ID NOs 7 to 31, or an allelic variant with up to 3 nucleotide analogues that differ in the sequence of a given oligonucleotide with respect to the corresponding PSA cDNA, or a salt thereof if salt-forming groups are present, or an oligonucleotide derivative thereof, or a salt thereof if salt-forming groups are present comprising at least one building block of formula I or I*,



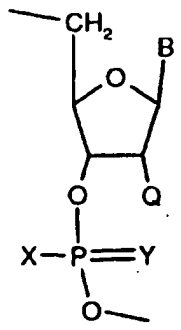
(I)



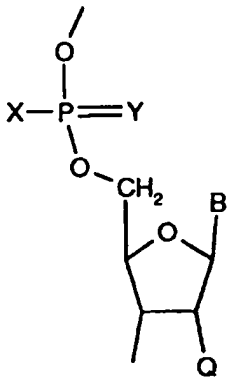
(I*)

wherein Q is H, OH, SH, SCH3, F, N_3, CN, OCN, OCH_3 or $O(CH_2)_zCH_3$, wherein z is from 1 to about 10, or $O(CH_2CHR_2O)_vR_1$, wherein R_1 is hydrogen, C_{1-21} -alkyl, C_{2-21}-alkenyl, or C_{2-21}-alkinyl, R_2 is hydrogen, C_{1-10} alkyl, or $-CH_2-O-R_3$, wherein R_3 is hydrogen, C_{1-20} -alkyl, or C_{2-20}alkenyl, and wherein v is from 1 to 4, and B is the base as defined in the given oligonucleotide sequence, or an analogue therof;

of formula IIa to IIf or IIa* to IIf*,



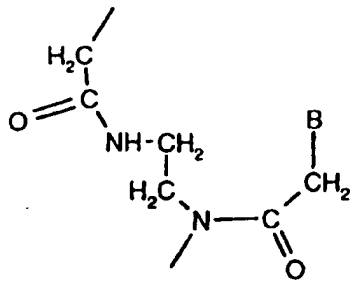
(IIa - IIf)



(IIa* - IIf*)

Radical of formula	type		
(IIa), (IIa*)	phosphorothioate	X = SH	Y = O
(IIb), (IIb*)	phosphorodithioate	X = SH	Y = S
(IIc), (IIc*)	methylphosphonate	X = CH_3	Y = O
(IId), (IId*)	phosphoramidate	X = NH-R	Y = O
(IIe), (IIe*)	boranophosphate	X = BH_3	Y = O
(IIf), (IIf*)	phosphotriester	X = O-R	Y = O

wherein R is lower alkyl;



(X*)

Radical of formula	type
X, X*	peptide nucleic acid

wherein B is a base radical as defined above, Q is H, OH, SH, SCH_3, F, N_3, CN, OCN, $O(CH_2)_zNH_2$ or $O(CH_2)_zCH_3$ wherein z is from 1 to about 10, $O(CH_2CH_2O)_vCH_3$, $O(CH_2CHR_2O)_vR_1$, wherein R_1 is hydrogen, C_{1-21} -alkyl, C_{2-21}-alkenyl, or C_{2-21}-alkinyl, R_2 is hydrogen, C_{1-10} alkyl, or $-CH_2-O-R_3$, wherein R_3 is hydrogen, C_{1-20} -alkyl, or C_{2-20}alkenyl, wherein v is from 0 to 12; and the other moieties have the meanings given behind the respective formula.

37. An oligonucleotide derivative according to claim 36 containing only phosphorothioate building blocks of formula IIa and/or IIa*, wherein X is SH and Y is O, the central group [O-(P-SH)(=O)-O] being tautomerizable to [O-(P=S)(-OH)-O] with the more stable form depending, among others, on the solvent and the state of ionization, and wherein B have the given meanings, i.e. the meanings set forth in the sequence listing, and Q being H, or a salt thereof.

38. An oligonucleotide derivative according to claim 36 wherein at least one building block (or more) belongs to the species of formula I or I*, or IIa or IIa*, in which formulas Q is $O(CH_2CHR_2O)_vR_1$, wherein R_1 is hydrogen, C_{1-21} -alkyl, C_{2-21}-alkenyl, or C_{2-21}-alkinyl, preferably hydrogen or methyl; R_2 is hydrogen, C_{1-10} alkyl, or $-CH_2-O-R_3$, wherein R_3 is hydrogen, C_{1-20} -alkyl, or C_{2-20}-alkenyl, R_2 preferably being hydrogen, methyl, - CH_2-OH, -or CH_2-OCH_3; wherein v is from 1 to 4, preferably from 1 to 3; B has the given meanings, i.e. the meanings set forth in the sequence listing, and all other intersugar linkages, i.e. those

which do not involve an above-defined modified sugar moiety, belong to the phosphorothioate type.

39. An oligonucleotide derivative according to claim 38 wherein the building blocks belong to the species of formula I or I*, in which formulas Q is $O(CH_2CHR_2O)_vR_1$, wherein R_1 is methyl, R_2 is hydrogen and v is 1, 2 or 3, or wherein R_1 is methyl or hydrogen, R_2 is methyl or - CH_2-OH, and v is 1.

40. An oligonucleotide derivative according to claim 36, which is a phosphorothioate type oligonucleotide derivative containing at least one amide type radical of formulas VIc, VIc*, VId, VId*, VII, VII*, VIII, or VIII*, preferably an amide- or amide III-type radical (formulas VIc, VIc* and VII, VII*, respectively), wherein B, X**, Y**, X_1 and Y_1 are as defined before, and Q is H, methoxy or methoxyethoxy ($CH_3OCH_2CH_2O$-).

41. An oligonucleotide derivative according to claim 36, which contains phosphodiester building blocks of formulas I or I* and at least one amide type radical of formulas VIc, VIc*, VId, or VId*, wherein B, X**, and Y** are as defined before, and Q is H, methoxy or methoxyethoxy (-OCH_2CH_2O CH_3).

42. A pharmaceutical composition that is suitable for administration to a warm-blooded animal suffering from a disease that responds to the modulation of PSA synthesis comprising an amount of a compound, particularly an oligonucleotide or oligonucleotide derivative, or of a salt thereof if salt-forming groups are present, according to claim 32, that is effective in the modulation of the synthesis of PSA, together with at least one pharmaceutically acceptable carrier.

43. A method of treating a disease that responds to the modulation of PSA synthesis comprising the administration of a PSA inhibitor, particularly an oligonucleotide or oligonucleotide derivative as defined in claim 32, or a pharmaceutically acceptable salt thereof, in an amount that is effective against the mentioned diseases to an animal in need of such treatment.

44. An oligonucleotide derivative, or a pharmaceutically acceptable salt thereof, according to claim 32 for the diagnostic or therapeutic treatment of a warm-blooded animal.

45. The use of an oligonucleotide derivative, or a pharmaceutically acceptable salt thereof, according to claim 32 for the preparation of a pharmaceutical composition for the treatment of tumor diseases that respond to the modulation of PSA synthesis.

46. A method of modulating the expression of PSA comprising contacting tissues or cells containing the PSA gene with an oligonucleotide or oligonucleotide derivative according to claim 32 containing the gene with an oligonucleotide derivative comprising from 5 to 50 nucleotide units specifically hybridizable with selected DNA or RNA deriving from the PSA gene.

47. A method of detecting the presence of DNA or RNA which encodes PSA in cells or tissues comprising contacting the cells or tissues with an oligonucleotide derivative according to claim 1 comprising from 5 to 50 nucleotide units specifically hybridizable with said DNA or RNA, and detecting if hybridization has occurred.

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12N15/57 C12N9/48 C07K16/40 C12N5/20 C12Q1/68
C12N5/10 C12Q1/37 A61K31/70 C07H19/167 C12N15/11

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 270, no. 45, 10 November 1995, MD US, pages 26931-26939, XP002020719 D. CONSTAM ET AL: "Puromycin-sensitive aminopeptidase"	1-35, 42-47
Y	see the whole document ---	36-41
Y	CHEMICAL REVIEWS, vol. 90, no. 4, 1 June 1990, pages 543-584, XP000141412 UHLMANN E ET AL: "ANTISENSE OLIGONUCLEOTIDES: A NEW THERAPEUTIC PRINCIPLE" see the whole document --- -/--	36-41

[X] Further documents are listed in the continuation of box C. [ ] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
"&" document member of the same patent family

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Date of the actual completion of the international search	Date of mailing of the international search report
19 February 1997	14.03.97

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+ 31-70) 340-3016	Van der Schaal, C